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(57) Abstract

A protein comprising the human Fas Ligand protein (hFasL) or a truncated human Fas Ligand protein or a functionally equivalent variant thereof that retain the Fas receptor-binding and apoptosis-inducing properties of hFasL and, linked either directly or indirectly to its C-terminus, a glycophospholipid is useful in preventing or treating tissue or organ graft rejection.

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Fas Ligand Fusion Protein

The present invention relates to a Fas Ligand protein-glycophospholipid fusion and to its use, e.g. to prevent rejection of tissue or organ transplants.

Fas Ligand (FasL) is a 40 kDa type II membrane protein that belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family and is expressed on immature thymocytes, activated T-cells, nonlymphoid cells in liver, ovary, heart etc. The nucleotide sequence and predicted amino acid sequence of rat FasL cDNA is disclosed by T. Suda et al. in Cell. 1993, Dec. 17, 75(6), 1169-78. SEQ ID No. 1 gives the amino acid sequences for human FasL (Takahashi et al., Intl. Immunol. 6, 1567-1574, 1994). The amino acid sequence of the intact protein is numbered from amino acid 1 to 281.

It is known that FasL interacts with the cell-surface receptor Fas expressed by certain tissue cells and induces apoptosis of these Fas antigen (sometimes also called Fas receptor) expressing cells. Mark R. Alderson et al. in J. Exp. Med., 181, 71-76, January 1995 disclose that activated mature T-cells express Fas antigen on their cell surface.

It is also known that endothelial cells expressing human FasL on their surface can, by interacting with the Fas antigen on cytotoxic T lymphocytes (CTL) induce apoptosis of these T-cells. T-cells being involved in graft rejection, it is desirable to obtain specifically apoptosis of the T-cells which attack the transplanted organ or tissue.

In accordance with the particular findings of the present invention, the present invention provides in a first aspect:

1. A hFasL protein-glycophospholipid fusion.

Such protein will incorporate its lipid tail into cell membranes, e.g. endothelial cell membranes, and thus present the FasL protein on the cell surface, e.g. to bind to Fas receptor present on other

cells and to thereby induce apoptosis of such other cells.

As used herein, the term "hFasL protein" encompasses full length human Fas Ligand protein, including the membrane-bound protein (comprising a cytoplasmic domain, a transmembrane region and an extracellular domain) as well as truncated human Fas Ligand proteins and functionally equivalent variants thereof that retain the Fas receptor-binding and apoptosis inducing properties of human Fas Ligand.

Characteristically the hFasL protein comprises at least the extracellular domain of human Fas-Ligand or a functionally equivalent part thereof or a functionally equivalent variant of these. Thus preferably the hFasL protein comprises the polypeptide having the amino acid sequence from position 103 to position 281 inclusive, more preferably from position 106 to position 281 inclusive, or most preferably from position 136 to position 281 inclusive, of the amino acid sequence shown in SEQ ID No. 2 or a functionally equivalent variant thereof.

For the purposes of the present description, a protein is functionally equivalent to the human Fas Ligand protein, if:

i. It has binding specificity for human Fas receptor similar to that of full length human Fas Ligand protein or the hFasL-GPI fusion protein as hereinafter described in the Examples, and

ii. it is capable, when present in as hFasL-glycophospholipid fusion protein, of inducing apoptosis of Fas receptor bearing cells, e.g. Lymphoma L1210-Fas cells, to a similar extent as the hFasL-GPI fusion protein as hereinafter described in the Examples, e.g. when tested in an in vitro assay as described in Example 3.

Also for the purposes of the present description, a protein is a variant of human Fas Ligand protein or of a part thereof, if the protein is at least 70%, preferably at least 80%, or more preferably at least 90% (especially at least 95%) homologous to the human Fas ligand amino acid sequence from position 1 to position 281 inclusive of SEQ ID No. 1 or the corresponding part thereof. In this context, amino acid sequences are at least 70% homologous to one another if they have at least 70% identical or conservatively

replaced amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions or non conservative substitutions in the amino acid sequences being counted as non-identical/non-conservatively replaced residues.

Further in this context and for the purposes of the present description conservative replacements may be made between amino acids in the following groups:

(i) alanine, serine and threonine;
(ii) glutamic acid and aspartic acid;
(iii) arginine and lysine;
(iv) asparagine and glutamine;
(v) isoleucine, leucine, valine and methionine, and
(vi) phenylalanine, tyrosine and tryptophan.

It will be appreciated, however, that conservative replacements may be inappropriate within critical regions of the sequence, such as an active site or binding site, and at the methionine coded by the start codon.

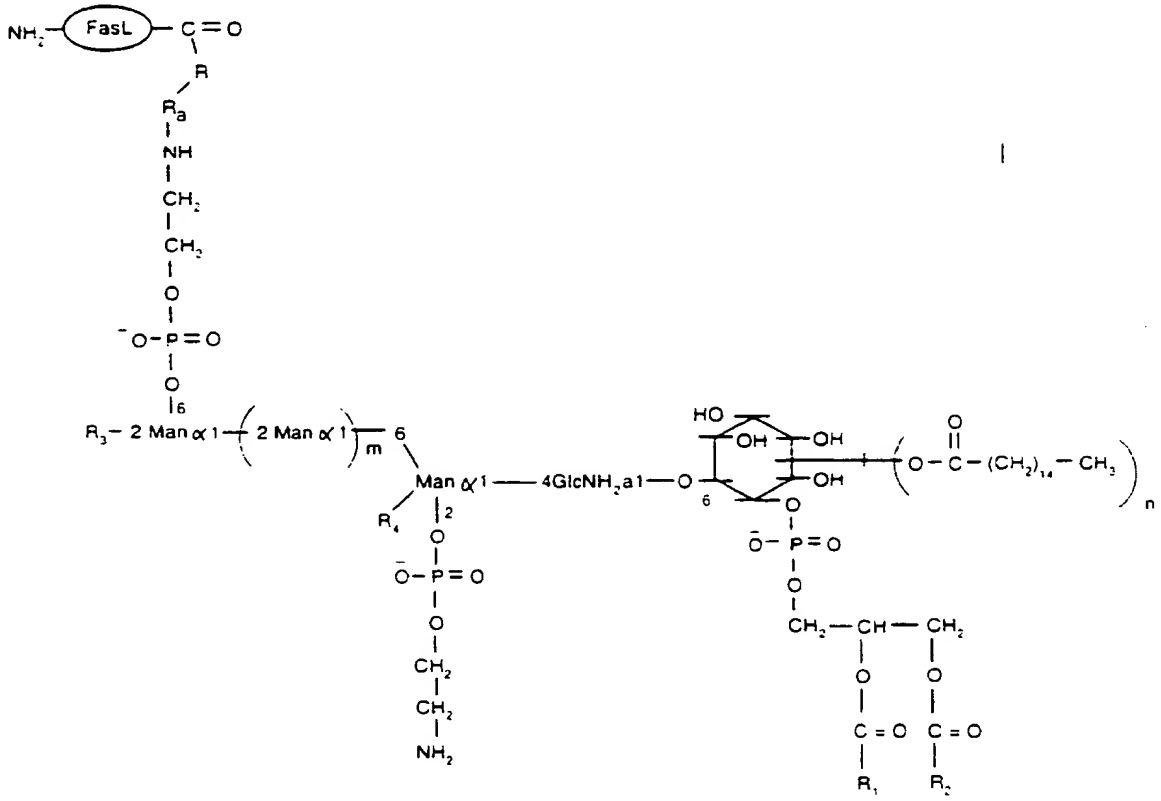
According to the invention, the hFasL protein is covalently linked by its C-terminal amino acid, either directly or indirectly to a glycophospholipid, preferably a glycosylated form of phosphatidyl-inositol, termed glycosyl-phosphatidylinositol (hereinafter GPI), e.g. as disclosed by M.P. Lisanti et al. in J. Membrane Biol. 117, 1-10, 1990.

In particular embodiments the C-terminal amino acid of the hFasL protein is linked by an amide bond either directly or via a linker to ethanolamine, which is in turn connected through a phosphodiester linkage to an oligo-saccharide of variable composition and structure. The terminal mono-saccharide of this glycan may be non-N-acetylated glucosamine which is linked at the C-1 position to the C-6 hydroxy of the inositol ring on phosphatidylinositol. The molecule may further comprise a glycerol lipid moiety which serves as the membrane-anchoring domain.

According to the invention, the GPI may be of any structure as present in naturally occurring GPI-linked proteins, e.g. hydrolytic enzymes such as alkaline phosphatase or acetylcholinesterase, mammalian antigens such as Thy-1, Thy-3, Ly-6, CD14 or CD16, protozoal antigens such as variant surface glycoprotein Trypanosoma, cell adhesion molecules such as LFA-3, or complement

molecules such as DAF.

In particular embodiments the FasL-glycophospholipid fusion protein of the invention may be represented by the formula I



wherein
m is 0 or 1,
n is 0 or 1,
R is a direct bond or a linker,
R_a is a direct bond or one or more amino acid residues derived from the selected GPI signal
each of R_1 and R_2, independently, is a fatty hydrocarbon residue, preferably C_{4-24}alkyl or C_{4-24}alkenyl, more preferably C_{12-22}alkyl or C_{12-22}alkenyl,
R_3 is H or 2 Manα1,
R_4 is H, Galα1-2 Galα1-^{6}Galα1- or 4βGalNAc1 when m is 1,

or R_4 is Galα1-6Galα1-2 Manα1 when m is 0, and
FasL being human FasL, a fragment thereof or a functionally equivalent variant thereof retaining the Fas-binding properties,

Gal being galactose, Man being mannose, GalNAc being N-acetyl-galactosamine and $GlcNH_2$ being non-N-acetylated glucosamine.

R may be a linker as used in the art in fusion proteins to link a C-terminal carboxy group to an amino group. Such a linker is preferably selected to provide flexibility to the protein, particularly to the extracellular domain of the protein. Examples of such linkers include e.g. a sequence of non polar amino acid units, e.g. 3 to 6 non polar α-amino acid units, preferably Gly and/or Ala units, e.g. -Gly-Gly-Gly-Gly-Gly-.

Protein fusions of the invention comprising a linker between the hFasL protein and the glycophospholipid moiety are encompassed by the expressions "hFasL protein-glycophospholipid fusion" and hFasL protein-GPI fusion as used hereinafter.

The hFasL protein-glycophospholipid fusions of the invention may be prepared synthetically by chemical linking of a hFasL protein and a glycophospholipid moiety, optionally in suitably protected followed by removal of protecting groups as required.

Conveniently, however, the hFasL protein-glycophospholipid fusion may be prepared by a recombinant DNA technology process involving expression of a protein comprising the hFasL protein amino acid sequence and post-translational modification of the expressed protein to yield the hFasL protein-glycophospholipid fusion. For such a process the expressed protein characteristically comprises a signal sequence, e.g. a c-terminal sequence of amino acid residues, which act as a trigger and site for post-translational modification of the expressed protein to give the hFasL protein-glycophospholipid fusion.

Thus in a further aspect the invention also provides a nucleotide sequence, e.g. a DNA sequence, coding for a protein comprising the amino acid sequence of a hFasL protein and a signal sequence for post translational modification of the protein to give a corresponding hFasL protein-glycophospholipid fusion, particularly hFasL protein-GPI fusion.

Preferably the nucleotide sequence is a DNA sequence suitable for eukaryotic or bacterial expression.

Thus the invention also provides an eukaryotic or bacterial expression vector comprising a DNA sequence coding for a protein comprising the amino acid sequence of a hFasL protein and a signal sequence for post translational modification of the protein to give a corresponding hFasL protein-glycophospholipid fusion, particularly hFasL protein-GPI fusion.

The expression vector typically contains, in addition to the protein coding sequence, appropriate expression control sequences including a suitable promoter, an operator and a ribosome binding site and other appropriate regulatory sequences. The expression vector may also contain one or more selectable markers. The promoter may be inducible by a variety of stimuli, e.g. exposure to a chemical or change in temperature. The promoter may also be cell-specific or cell cycle specific.

The promoter may be any promoter which is active in E.coli, e.g the bacteriophage T7 promoter, and the expression vector may be a plasmid. For E.coli expression a R1 or CO1-E1 plasmid-derived vector may be used. For eukaryotic expression, a vector containing a viral promoter, e.g. based on pXMT2 or pXMT3 may be employed. The invention also provides bacterial or eukaryotic host cells transformed with a hFasL protein glycophospholipid fusion, particularly the hFasL fusion GPI protein, coding sequence or an expression vector as described above. Any suitable bacterial host may be used, preferably E. coli. A suitable eukaryotic host is COS cells for transient and CHO cells for stable expression.

Attachment of the glycophospholipid, e.g. GPI, moiety is a post-translational modification which conveniently occurs in the endoplasmic reticulum (ER) of the host cell. As a result of glycophospholipid (e.g. GPI) addition a hydrophobic sequence is typically removed from the carboxy terminus of the nascent protein. Such an hydrophobic sequence is often a necessary portion of the post-translational modification signal sequence. A CAS doublet (cleavage/attachment site), e.g. Ser-Ser, Ser-Gly, Ser-Ala, in conjunction with a hydrophobic carboxy terminus often provides the minimal sequence necessary for glycophospholipid, e.g. GPI, addition. A spacer of 5-20, more preferably 7-14 amino acids may be desirable between the FasL protein sequence and the post-translational modification signal sequence. The hydrophobic domain conveniently functions in the ER to slow or temporarily stop the

transit of the nascent protein through the membrane of the ER so that attachment of the GPI moiety can occur.

Provided the expressed protein comprises an appropriate post-translational modification signal sequence, addition of glycophospholipid, e.g. GPI, occurs broadly in most host cells.

The DNA coding for the protein comprising the amino acid sequence of the hFasL protein and the post-translational modification signal sequence may be prepared by appropriate ligation of hFasL protein coding sequence and DNA sequence coding for the signal sequence.

In a particular embodiment, a hFasL protein-GPI fusion may be prepared by a process which comprises:

a) generating a GPI addition signal sequence e.g. using two overlapping oligonucleotides which are filled in using a polymerase. Each of the 5' end and 3' end contains a restriction site. The filled in product is cloned into an expression vector, e.g. PXMT3.

b) using a clone containing the human Fas-ligand cDNA, e.g. as a template, to amplify the extracellular domain of human Fas-ligand, e.g. the fragment comprising amino acids 136 to 281 of the human Fas-ligand sequence. The 3' oligonucleotide may be designed so that it encodes the desired linker and a restriction site at it's 3' end. The 5' oligonucleotide preferably does not contain any restriction site but overlaps the signal sequence of human Fas by several nucleotides, e.g. 14 nucleotides. The human Fas signal sequence may be generated using two oligonucleotides which are filled in with a polymerase. The noncoding oligonucleotide may overlap Fas-ligand by several nucleotides, e.g. 10 to 22, preferably 22 nucleotides. The Fas-ligand PCR product and the filled in Fas signal sequence are typically spliced, e.g. using the overlap PCR technique, digested and cloned into the plasmid containing the GPI signal sequence. The corresponding hFasL-GPI construct is indicated in Fig. 1.

Purification or isolation of hFasL-glycophospholipid fusion protein particularly hFasL-GPI, may be accomplished by any method known in the art that does not result in substantial degradation of the

fusion protein. Suitable methods are e.g. affinity chromatography, immunoaffinity chromatography, HPLC and FPLC.

The hFasL-glycophospholipid fusion protein of the invention, particularly the hFasL-GPI fusion protein is useful for inducing Fas-mediated cell death, particularly T-lymphocyte death. Where cells of a tissue for transplantation bear on their surfaces foreign histocompatibility antigens, these antigens cause cytotoxic T-lymphocyte activation in recipients, leading to donor cell destruction after several sequential activation steps. The hFasL protein-glycophospholipid fusion, particularly the hFasL protein-GPI fusion may be useful to treat acute graft rejection. A conventional route of therapy for acute graft rejection results in severe immunosuppression in the recipient host. Treatment with the hFasL protein-GPI fusion should provide a more specific treatment for activated T-lymphocytes, i.e. for T-lymphocytes expressing the Fas antigen which attack the transplanted tissue or organ, not all the T-lymphocytes present in the immune system.

The hFasL protein-glycophospholipid fusion, particularly the hFasL protein-GPI fusion, may also be useful to treat chronic transplant rejection, including both allograft and xenograft rejection.

In a further embodiment the invention provides:

2. A process for incorporating a hFasL protein-glycophospholipid fusion, particularly hFasL protein-GPI fusion, into endothelial cells of a tissue or an organ, which process comprises infusing the organ or incubating a tissue with a purified hFasL protein-glycophospholipid fusion, particularly hFasL protein-GPI fusion.

3. A method for inducing Fas-mediated death of endothelial cells at a targeted tissue or organ comprising infusing the targeted organ or incubating the targeted tissue with a purified hFasL protein-glycophospholipid fusion, particularly hFasL protein-GPI fusion.

4. A method for preventing or treating tissue or organ allograft or xenograft rejection in a subject which comprises infusing the donor organ or incubating the donor tissue, with a purified hFasL protein-glycophospholipid fusion, particularly hFasL

protein-GPI fusion prior to transplantation.

In the methods of the invention as defined under 3 to 4 above, the protein fusion of the invention is particularly useful in preventing symptoms associated with acute or chronic organ or tissue allo- or xenograft transplant rejection, e.g. heart, lung, combined heart-lung, liver, kidney, pancreatic (complete or partial, e.g. Langerhans islets), skin, corneal transplants or bone marrow, particularly transplant vasculopathies, e.g. graft atherosclerosis.

As alternatives to the above, the present invention also provides:

5. A hFasL protein-glycophospholipid, particularly hFasL protein-GPI fusion for use in any method as defined under 2 to 4 above; or

6. A hFasL protein-glycophospholipid, particularly hFasL protein-GPI fusion for use in the preparation of a pharmaceutical composition for use in any method as defined under 2 to 4 above; or

7. A composition for use in any method as defined under 2 to 4 above comprising a hFasL protein-glycophospholipid, particularly hFasL protein-GPI fusion, together with one or more pharmaceutically acceptable diluents or carriers therefor.

The following examples are given by way of illustration and are not to be construed as limiting the invention in any way inasmuch as many variations of the invention are possible within the spirit of the invention.

Example 1: Plasmid Construction and Cloning of the DNA encoding hFasL-GPI fusion protein

The GPI addition signal sequence derived from human CD16 is generated using two overlapping oligonucleotides which are filled in using Klenow polymerase. The 5' end contains a PstI and a SpeI site, the 3' end an EcoRI site. The filled in product is phosphorylated (using T4 Kinase), gel purified and ligated into PstI/EcoRI digested PXMT3 expression vector.

Oligonucleotides:

Pst Spel
GPI5': GTC ACT AGT TTG GCA GTG TCA ACC ATC TCA TCA TTC TCT
CCA CCT GGG TAC CAA GTC TCT TTC TGC TTG GTG ATG GTA
(SEQ ID No. 3)

EcoRI
GPI3": GTC GAA TTC TCA AAT GTT TGT CTT CAC AGA GAA ATA TAG
TCC TGT GTC CAC TGC AAA AAG GAG TAC CAT CAC CAA GCA
GAA (SEQ ID No. 4)

A clone containing the human Fas-ligand cDNA is used as a template to amplify the extracellular domain of human Fas-ligand comprising amino acids 136 to 281 of the published human Fas-ligand sequence. The 3' oligonucleotide (Fas4) is designed so that it encodes for additional 6 glycine residues and a SpeI site at it's 3' end. The 5' oligonucleotide (Fas3) does not contain any restriction site but overlaps the signal sequence of human Fas by 14 nucleotides. The human Fas signal sequence is generated using two oligonucleotides (Fas1, Fas2) which are filled in with Klenow polymerase. The noncoding oligonucleotide overlaps Fas-ligand by 22 nucleotides. The Fas-ligand PCR product and the filled in Fas signal sequence are spliced using the overlap PCR technique, gel purified, digested with PstI and SpeI and cloned into similarly digested above described plasmid containing the GPI signal sequence.

Oligonucleotides:

PstI
FAS1: TCT CTG CAG ATG CTG GGG ATC TGG (SEQ ID No. 5)

FAS2: GGG TGG AGC AAC AGA CGT AAG AAC CAG AGG TAG GAG GGT CCA GAT GCC CAG CAT CTG CAG AGA (SEQ ID No. 6)

FAS3: TTA CGT CTG TTG CTC CAC CCC CTG AAA AAA AGG AG (SEQ ID No. 7)

SpeI
FAS4: CAA ACT AGT GCC ACC ACC GCC TCC ACC GAG CTT ATA TAA GCC GAA AAA CG (SEQ ID No. 8)

The entire construct is sequenced using the T7 sequencing kit from Pharmacia Biotech (Cat. No. 27-168201).

Example 2: Purification

The recombinant fusion protein is purified using a Fas-Fc affinity column (or an anti FasL antibody affinity column) as described by T. Suda and S. Nagata in J. Exp. Med. **179**: 873-879, 1994. An endotoxin free fusion protein is obtained.

Example 3: Transient expression of hFasL-GPI in COS cells

$9x10^5$ cells are seeded in DMEM, 10% FCS on a 60 mm plate and incubated over night at 37°C, 5% CO_2. Cells are calcium phosphate transfected with 6 µg of plasmid DNA (hFasL-GPI) using the ProFection Mammalian Transfection System (Promega). As shown in Fig. 2, cells are analyzed by FACS 36 h after transfection using an anti-human Fas-ligand primary monoclonal antibody (clone NOK-1; Pharmingen) and a phycoerythrin labeled anti mouse IgG secundary antibody. PXMT3 mock transfected COS cells are used as negative controls, COS cells transfected with a construct containing human Fas-ligand cDNA are used as positive controls (Fig. 3).

Fig. 2: FACS analysis of COS cells transiently transfected with hFasL-GPI expression construct

Fig. 3: Positive and negative controls

The infusion or the incubation with a hFasL-glycophospholipid fusion protein according to the invention may advantageously be performed at a temperature of about 4° to 37° C. Preferably it is carried out for a duration period of about 2 to 20 hours. The hFasL-glycophospholipid fusion protein of the invention may be added to a solution or preparation as usually used for storing donor tissue or a donor organ prior to transplantation, e.g. a so-called "University of Wisconsin solution". Alternatively, the fusion protein may also be used in saline optionally buffered, e.g. phosphate buffered saline, or in physiologically solution. The concentration of hFasL-glycophospholipid may vary; it may advantageously be 10-40 mg/ml of infusion or incubation solution.

Utility of the hFasL-glycophospholipid fusion protein of the invention may be demonstrated for example in accordance with the methods hereinafter described.

In vitro Assay

COS cells are transiently transfected with the construct of Example 1 or with a control construct encoding the entire hFasL protein (without glycophospholipid moiety). This leads to incorporation into the cell membrane via transmembrane domain and expression on the cell surface. The apoptotic effect of above mentioned cells, native COS cells and COS cells incubated with purified hFasL-GPI on Cr labeled lymphoma L1210 and lymphoma L1210-FAS are compared (Lymphoma cell lines: Schulz M. et al. Eur. J. Immunol. **25**: 474-480, 1995). Cell death of Lymphoma L1210-Fas is significantly higher with COS cells transfected with the construct of Example 1 due to Fas-L induced apoptosis.

In vivo Assays

A. Mouse heart infusion

Mouse hearts are infused with a University of Wisconsin solution (Eurocollins solution) comprising 25 mg/ml of hFasL-GPI for 8 hours at 37°C. Paraffin sections of the hearts are probed with biotinylated hFasL antibodies for assessment of the hFasL coating.

B. Vascularized heterotopic heart transplantation

Following heparinization and simultaneous exsanguination of the donor animal, the thorax is opened and packed with ice. The donor heart is prepared by ligation and division of the superior vena cava, inferior vena cava, left pulmonary artery and right pulmonary veins. The aorta is ligated and divided distal to the branchiocephalic trunk which is divided at the first bifurcation (right common carotid and subclavian arteries). Additional cold heparinized saline is infused via the brachiocephalic stump. The organ is infused with recombinant hFasL-GPI fusion protein of Example 2. Remaining pulmonary veins are ligated in one ligature and the heart removed into cold saline.
The hearts are implanted onto the recipients abdominal vessels: brachiocephalic trunk to aorta and right pulmonary artery to inferior vena cava with end-to-side anastomoses using 11/0 Ethilon (Ethicon, Norderstedt, Germany) continuous sutures. Animals are closed in two layers with 6/0 Vicryl (Ethicon) and kept warm until fully recovered. Total ischaemia times are in the range of 40-50 min of which 25-35 min are at 4°C. During anastomosis (10-15 min) the graft is kept cold.
After transplantation, graft function is monitored by daily assessment of graft beat (palpation). Rejection is considered to be complete when heart beat stopps. In all experiments rejection is confirmed by histological examination of the grafts.
Significant improvements are obtained with hearts infused with hFasL-GPI fusion protein prior to transplantation compared with control animals (no hFasL-GPI infusion prior to transplantation).

The use of the fusion protein of the invention to prevent or treat graft rejection may be combined with an immunosuppressive treatment, e.g. administration of an immunosuppressive agent to the recipient after transplantation such as cyclosporin A, cyclosporin G, FK 506, leflunomide or an analogue thereof, mizoribine, mycophenolic acid, mycophenolate mofetil, immunosuppressive monoclonal antibodies, e.g. monoclonal antibodies to leucocyte receptors, e.g. MHC, CD2, CD3, CD4, CD7, CD25, CD28, CTLA4, B7, CD45 or CD58 or their ligands.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Fas Ligand Fusion Protein

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp
1               5                  10                  15

Ser Ser Ala Ser Ser Pro Trp Ala Pro Pro Gly Thr Val Leu Pro Cys
            20                  25                  30
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Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro
        35                  40                  45

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro
    50                  55                  60

Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly Asn His Ser Thr Gly
65                  70                  75                  80

Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly
                85                  90                  95

Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala
            100                 105                 110

Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu
        115                 120                 125

Lys Gln Ile Gly His Pro Ser Pro Pro Pro Glu Lys Lys Glu Leu Arg
    130                 135                 140

Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu
145                 150                 155                 160

Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr
                165                 170                 175

Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr
            180                 185                 190

Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser
        195                 200                 205

His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met
    210                 215                 220

Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala
225                 230                 235                 240

Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His
                245                 250                 255

Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser
            260                 265                 270

Gln Thr Phe Phe Gly Leu Tyr Lys Leu
        275                 280
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Pro Pro Pro Glu Lys Lys Glu Leu Arg Lys Val Ala His Leu Thr Gly
1 5 10 15

Lys Ser Asn Ser Arg Ser Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly
20 25 30

Ile Val Leu Leu Ser Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Ile
35 40 45

Asn Glu Thr Gly Leu Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly
50 55 60

Gln Ser Cys Asn Asn Leu Pro Leu Ser His Lys Val Tyr Met Arg Asn
65 70 75 80

Ser Lys Tyr Pro Gln Asp Leu Val Met Met Glu Gly Lys Met Met Ser
85 90 95

Tyr Cys Thr Thr Gly Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala
100 105 110

Val Phe Asn Leu Thr Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu
115 120 125

Leu Ser Leu Val Asn Phe Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr
130 135 140

Lys Leu
145

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTCACTAGTT TGGCAGTGTC AACCATCTCA TCATTCTCTC CACCTGGGTA CCAAGTCTCT 60

TTCTGCTTGG TGATGGTA 78

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTCGAATTCT CAAATGTTTG TCTTCACAGA GAAATATAGT CCTGTGTCCA CTGCAAAAAG 60

GAGTACCATC ACCAAGCAGA A 81

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TCTCTGCAGA TGCTGGGGAT CTGG 24

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGGTGGAGCA ACAGACGTAA GAACCAGAGG TAGGAGGGTC CAGATGCCCA GCATCTGCAG 60

AGA 63

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TTACGTCTGT TGCTCCACCC CCTGAAAAAA AGGAG 35

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

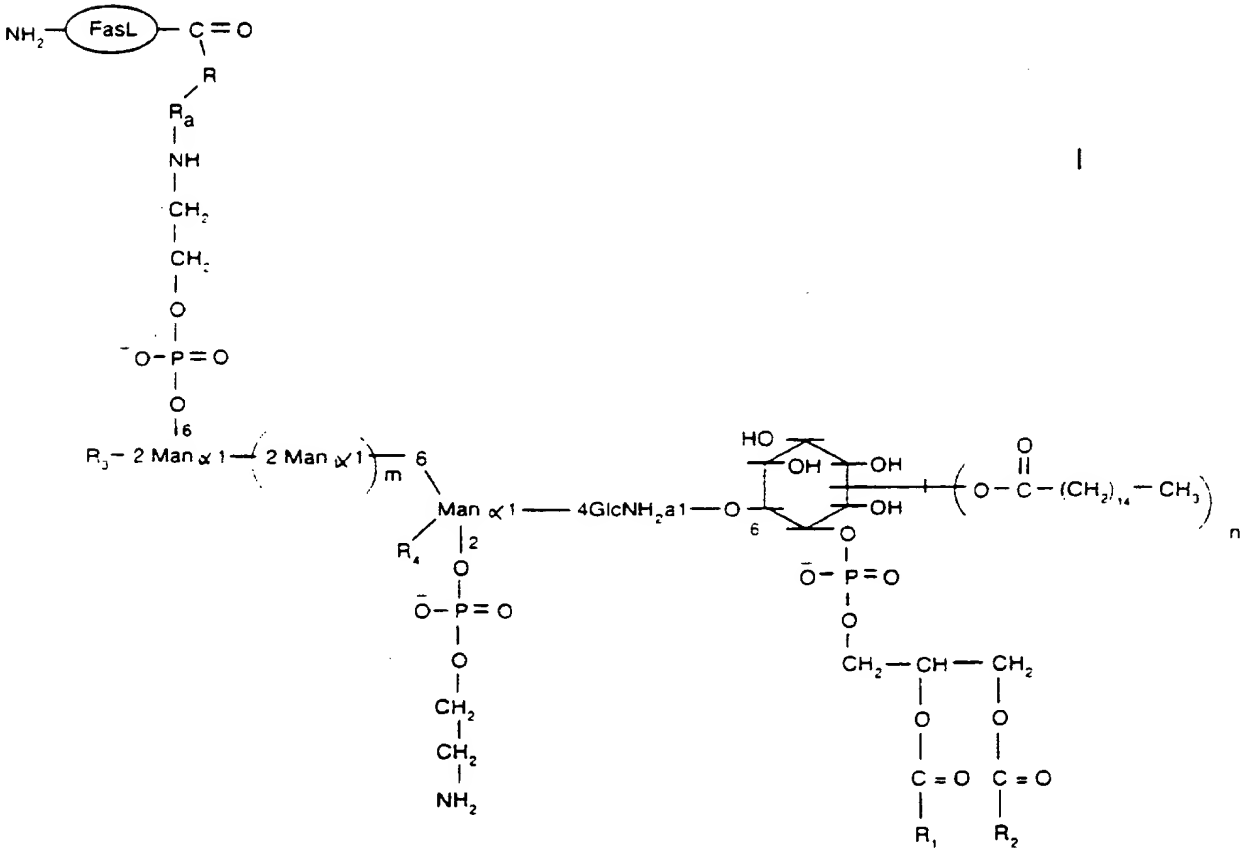
CAAACTAGTG CCACCACCGC CTCCACCGAG CTTATATAAG CCGAAAAACG 50

CLAIMS

1. A protein comprising
   - the human Fas Ligand protein (hFasL) or a truncated human Fas Ligand protein or a functionally equivalent variant thereof that retain the Fas receptor-binding and apoptosis inducing properties of hFasL and, linked either directly or indirectly to its C-terminus,
   - a glycophospholipid.

2. A protein according to claim 1 wherein the glyco-phospholipid is glycosyl-phosphatidyl inositol (GPI).

3. A protein according to claim 1 of formula I



I

wherein
m is 0 or 1,
n is 0 or 1,
R is a direct bond or a linker,

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 96/05039

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, vol. 75, December 1993, pages 1169-1178, XP000579690 SUDA T. ET AL.: "Molecular cloning and expression of the Fas Ligand, a novel member of the tumor necrosis factor family." cited in the application see the whole document ---	1-10
A	J. EXP. MED., vol. 181, January 1995, pages 71-77, XP000645126 ALDERSON M. ET AL. : "Fas ligand mediates activation-induced cell death in human T-lymphocytes." cited in the application see the whole document ---	9
A	SCIENCE, vol. 267, 10 March 1995, pages 1449-1456, XP002026333 NAGATA S. AND GOLSTEIN P.: "The Fas death factor." see the whole document -----	1-10

3

INTERNATIONAL SEARCH REPORT

.formation on patent family members

Intern al Application No
PCT/EP 96/05039

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2179909 A	13-07-95
		EP 0739350 A	30-10-96
US 5223408 A	29-06-93	NONE	